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Environmental levels of atrazine and its degradation products impair survival skills and growth of red drum larvae

María del Carmen Alvarez*, Lee A. Fuiman

University of Texas at Austin, Marine Science Institute, Port Aransas, TX 78373, USA

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Abstract

Red drum larvae (*Sciaenops ocellatus*) were exposed to environmentally realistic and sublethal levels of the herbicide atrazine (2-chloro-4-ethylamin-6-isopropylamino-*S*-triazine) to evaluate its effects on ecologically critical traits: growth, behavior, survival potential, and resting respiration rate. Settlement size larvae (7 mm total length) were given an acute exposure of atrazine at 0, 40, and 80 $\mu g\,l^{-1}$ for 4 days. Tests of 96 h survival confirmed that these naturally occurring concentrations were sublethal for red drum larvae. Growth, routine swimming, antipredator responses to artificial and actual predators, and resting respiration rate were monitored 1 and 3 days after onset of exposure. Atrazine exposure significantly reduced growth rate. Atrazine-exposed larvae also exhibited significantly higher routine swimming speeds, swam in more convoluted paths, and were hyperactive. Responses to artificial and actual predators were not affected by atrazine exposure nor were resting respiration rates. The higher rate of travel (86% higher in atrazine-treated larvae) resulted in higher predicted encounter rates with prey (up to 71%) and slow moving predators (up to 63%). However, hyperactivity and faster active swimming speeds of exposed larvae indicated that naturally occurring sublethal levels of atrazine will result in an elevated rate of energy utilization (doubling the total metabolic rate), which is likely to increase the risk of death by starvation. Moreover, atrazine effects on growth will prolong the larval period, which could reduce the juvenile population by as much as 24%. We conclude that environmentally realistic levels of atrazine induce behavioral and physiological effects on fish larvae that would compromise their survival expectations.

Keywords: Atrazine; Fish larvae; *Sciaenops ocellatus*; Behavior; Respirometry; Metabolism; Endocrine disrupting chemical

1. Introduction

Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-*S*-triazine) is a widely used herbicide in the United States. It was first registered in 1958 and the U.S. Environmental Protection Agency has estimated that between 34 and 36 million kilograms of atrazine were used in 2001 (Kiely et al., 2004). Due to its high use and its relatively high mobility in soils, atrazine is frequently detected in surface and ground waters. Atrazine levels in runoff can reach very high levels in the first rain events after application (Southwick et

* Corresponding author. Tel.: +1 361 749 6775; fax: +1 361 749 6777.
E-mail address: mdcalvarez@yahoo.com (M. del Carmen Alvarez).

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al., 2003). Thurman et al. (1992) showed that atrazine levels in storm runoff could reach levels of 40 μg l^{-1}. Reported levels in South Texas coastal waters have reached 65 μg l^{-1} (Pennington et al., 2001).

Commercially and ecologically important fish species such as red drum (*Sciaenops ocellatus*) can be affected by contaminated runoffs entering estuaries. Red drum spawn in coastal areas and they reach the estuaries still in the larval stage. Contaminants in the environment can impair growth and development of larvae and may ultimately lead to mortality (e.g., Weis and Weis, 1976, 1995; Faulk et al., 1997; Zhou and Weis, 1998; Beg et al., 2001; McCarthy et al., 2003). Although atrazine was developed to inhibit photosynthesis in plants, it has multiple effects on animals. For example, atrazine is a classified endocrine disrupting chemical that affects steroidogenesis in alligators and frogs (Crain et al., 1997; Hayes et al., 2002; Goulet and Hontela, 2003) and olfactory-mediated endocrine function in salmon parr (Moore and Lower, 2001). Atrazine exposure has been shown to produce altered social and antipredator behavior in goldfish (*Carassius auratus*) (Saglio and Trijasse, 1998). This study assesses the effects of environmentally realistic levels of atrazine on red drum larvae at the size they enter contaminated nursery areas by evaluating ecologically important behaviors, growth, and the energetic cost of exposure.

2. Materials and methods

2.1. Experimental animals

Red drum eggs were obtained from three sources: University of Texas Fisheries and Mariculture Laboratory (Port Aransas, TX), Texas Parks and Wildlife Department CCA/CPL Marine Development Center (Corpus Christi, TX), and Perry R. Bass Marine Fisheries Research Station (Palacios, TX). Broodstocks were induced to spawn by manipulating ambient temperature and photoperiod (Arnold, 1988). Eggs were collected within 12 h of spawning and hatched in conical tanks in 20 l of sea water. From day 5 after hatching, the volume was gradually increased to 100 l over approximately a 5 d. Larvae were reared with flow-through circulation until experimentation. Temperature and salinity in the rearing tanks were maintained at about 27 °C and 27 PSU. Larvae were fed 10 rotifers (*Brachionus plicatilis*) ml^{-1} d^{-1} from day 1 until about 10 d posthatching when their diet was gradually shifted to *Artemia* nauplii over a 3 d period. At the time of the chemical exposure fish were completely weaned from rotifers and onto nauplii. *Artemia* were enriched overnight with Algamac 2000 (Aquafauna Bio-Marine Inc., CA) and added to rearing tanks in the morning so that they reached a concentration of 5 nauplii ml^{-1}. Fish were fed at approximately 08:30 h daily and allowed to feed for about 1 h before moving them to the experimental chambers.

Since the highest levels of atrazine in surface waters are found in estuarine areas, exposures were conducted on larvae at the size of settlement to estuarine seagrass beds, about 7–8 mm total length (TL) (Rooker and Holt, 1997; Herzka et al., 2002). Red drum larvae reached settlement size within 15–20 days under rearing conditions.

2.2. Exposures

Atrazine (with a guaranteed purity of 98%) was purchased from Chem Service Inc. (West Chester, PA). Atrazine (approximately 24 mg) was dissolved in 3 ml of acetone and added with gentle stirring to the trial tanks to the desired concentration. An equal amount of acetone was also added to the control group.

Survival experiments were performed to evaluate whether environmentally realistic doses were within the sublethal range for settlement size red drum. Groups of 50 settlement size larvae were transferred to 1.5-l exposure watch bowls (20 cm diameter) in a temperature-controlled room. Salinity and temperature were maintained at 27 °C and 27 PSU. Atrazine dissolved in acetone or acetone alone was added to the watch bowls 24 h after transfer. Doses tested were 0, 40, 80, and 500 μg atrazine l^{-1}. Fish were fed a ration of 5 nauplii ml^{-1} d^{-1}. Survival was recorded 96 h after the herbicide was added to the water. Six replicates for 40 and 500 μg l^{-1} exposures, and 12 for control and 80 μg l^{-1} exposures were performed with larvae from five different spawns. The proportions of surviving larvae from the atrazine-exposed groups were compared to the survival rate of control groups.

Settlement-size red drum larvae (7–8 mm TL) were transferred to six 50-l fiberglass tanks on experimental day −1 at a density of 10 larvae l^{-1} and allowed to recover from handling for 1 d. The six tanks were randomly assigned to the three treatment groups: control, low, and high dose (0, 40, and 80 μg of atrazine l^{-1}, respectively) in duplicate. Atrazine was added (experimental day 0) as described above. The exposure tanks were arranged in a water bath to minimize variations in temperatures among tanks. Mean (±S.D.) water temperature and salinity were $28.4 \pm 0.67\,^{\circ}C$ and 27.1 ± 0.14 PSU.

Water samples were taken 5 min (0 h) and 96 h after the addition of atrazine and sent for analysis to an independent laboratory (Department of Soil and Crop Sciences, Pesticide Fate Research Laboratory, Texas A&M University, College Station, TX) to characterize the exposures. Concentrations of atrazine and several degradation products (diamino-, deisopropyl-, hydroxy-, and desethyl-atrazine) were measured using high-performance liquid chromatography (HPLC), as described in Senseman et al. (1997).

2.3. Growth

Ten spawns were used for analysis of growth rates. Growth rates were calculated over a 9 d period. Groups of 20 larvae were sampled from each exposure tank on the day of transfer (experimental day −1) and on days 1, 3, 6, 7, and 8 after atrazine was added to the water. Fish were anesthetized using tricaine methansulfonate (MS 222) and photographed along with a reference scale using a digital camera (Sony DCR-TRV350) attached to a dissecting microscope. Total length (mm) was measured using an image-processing program (ImageJ, National Institutes of Health). Ten replicates from 10 different spawns were done.

Because larval growth rate is size-dependent, growth rate was calculated using an exponential growth model to compensate for differences in initial size among trials:

$$\mathrm{TL}_t = \mathrm{TL}_{-1}\mathrm{e}^{Gt}$$

where TL_t total length (mm) on experimental day t, TL_{-1} is total length on experimental day −1, and G is the instantaneous growth rate (d^{-1}).

2.4. Behavioral experiments

Two behavioral assays, routine swimming and visual startle response, were conducted on larvae from four spawns to assess the effects of atrazine exposure. Routine swimming behavior measures foraging capacity and the visual startle response measures the ability of larvae to escape from a predatory attack (Fuiman, 1994).

Groups of 10 larvae were randomly selected from each exposure tank and carefully placed into glass chambers (75 mm × 70 mm × 20 mm) containing approximately 50 ml of filtered sea water from their exposure tank. The chambers were placed in a temperature-controlled room and the fish were left to recover from handling for 2–3 h (Fuiman and Ottey, 1993). After this time, the chamber was carefully placed above a video camera (Cohu, model 3315-2000/0000) and left undisturbed for 5 min to allow the larvae to acclimate. Routine behavior of the undisturbed larvae was then video-recorded (Panasonic AG-1960) from a remote station for 3 min. After this time, the larvae were given an artificial predatory visual stimulus and their responses were recorded (for details see, Fuiman and Cowan, 2003). The recorded video was digitalized as AVI files and movements of the larvae were analyzed with the aid of a computerized tracking system (WinAnalyze 2D Software, Version 1.5, Mikromak, Germany).

Analysis of the routine behavior clips was conducted frame by frame and the paths described by the larvae were tracked throughout 25 s. The behavior was measured for all 10 larvae in the chamber and expressed by four variables: (1) rate of travel (mm s^{-1}), (2) active swimming speed (mm s^{-1}), (3) activity (% time), (4) net-to-gross displacement ratio (NGDR, dimensionless). Red drum larvae swim in alternating episodes of active swimming and resting. Rate of travel is the average swimming speed (mm s^{-1}) including the resting periods. Active swimming speed (mm s^{-1}) is the average velocity during active time only. Activity is the percentage of time the larva is actively swimming. NGDR expresses the linearity of the path described by a larva. Net displacement is the straight-line distance between the starting point and the ending point of the video segment analyzed. Gross displacement is the actual distance covered by the larva along its swimming path. Therefore, the closer NGDR approaches 1.0, the more linear the swimming path.

Frame-by-frame analysis of the visual response assays began 50 video fields before the time when the pendulum reached its nearest position to the chamber and ended 50 fields after (total: 100 frames or 1.7 s). Responses were characterized by: (1) responsiveness, percentage of larvae responding to the stimulus; (2) maximum response speed (mm s^{-1}); (3) average response speed (mm s^{-1}); (4) latency, elapsed time between the release of the stimulus and the larval response (ms); (5) time to maximum speed (ms).

2.5. Predator exposure

Groups of 20 larvae from each exposure tank were used in four replicate spawns. Experiments were run in duplicate for each spawn. Larvae and a predator (50–60 mm gulf killifish, *Fundulus grandis*) were confined at opposite sides of a 45-l aquarium (filled with 15 l of sea water) in transparent plastic cylinders of 4 and 15.5 cm diameter, respectively. The video camera was positioned over the cylinder containing the larvae with a field of view of about half of the aquarium (25 cm × 25 cm). Predator and prey were allowed to acclimate from handling for 2–3 h (Fuiman and Ottey, 1993). Following this, the video recorder was started and the cylinder containing the larvae was raised gently, freeing them into the tank. Immediately afterward the predator was released in the same way. No shelter was provided for the larvae and experiments were run until all of the larvae were eaten or disappeared from the field of view. Encounters within the video camera's field of view were recorded for later frame-by-frame analysis. Each attack, response, capture, escape, and secondary attack (see explanation below) was recorded as a binomial variable, given a value of 1 if the event occurred and 0 if it did not. Calculations of responsiveness to an attack, response effectiveness, prey error, and capture success were made from the binomial variables. Responsiveness was the percentage of fish in a trial responding to a predator attack. Response effectiveness was the number of fish responding to attack but not captured. Some fish initiated a startle response in the proximity of the predator even though they were not attacked. In many cases, this behavior made the larva conspicuous to the predator triggering an attack (secondary attack). Prey error represents the proportion of false alarms triggering a secondary attack. Finally, capture success is the total proportion of attacked larvae captured.

2.6. Respiration rates

The energetic cost of atrazine exposure was evaluated in a specially designed respirometer. The respirometer had four independent chambers, each one with a total volume of 14 ml and consisting of an upper loop (Fig. 1) with a length of 36 mm connected by three-way valves to a lower loop of about 30 mm in length containing the fish compartment (30 mm × 11 mm). These valves controlled whether the system was in recirculating or flow-through mode. A larva was contained within the fish compartment by a 500-μm mesh placed at each end of the compartment. Water used for the experiments was previously autoclaved to minimize bacterial oxygen consumption and then fully oxygenated. The oxygen sensor was a flow-through cell enclosing a fiber-optic oxygen micro-sensor with optical isolation (PreSens GmbH, Germany). Oxygen measurements (% of air saturation) were done with temperature compensation at 23.6 ± 0.6 °C and a salinity of 25 PSU. The chambers were immersed in a water bath to minimize temperature differences.

In the early afternoon of each experimental day, three to four larvae from each treatment were randomly selected and transferred to a glass dish (6 cm diameter) with 20 ml of filtered sea water from their original tanks. Fish were left undisturbed for 6 h to completely evacuate their guts after which a single fish was carefully transferred to the fish compartment. Each trial consisted of four separate chambers, three containing one experimental fish (one from each treatment) and a fourth chamber was left empty to control for background bacterial respiration. The chambers were then placed in flow-through mode for 2 h to allow the fish to recover from handling, reduce stress-related increase in oxygen consumption and maintain high levels of dissolved oxygen. After acclimation, the valves were adjusted to place the system into recirculation mode and oxygen measurements were started. Water flow within the chambers was maintained at a speed of approximately 1 mm s^{-1} with a peristaltic pump (Masterflex® L/S™, model 7519-15). Continuous measurements of oxygen content were conducted on 12 fish of each treatment and on 11 controls (all from separate spawns) for a period of 5–7 h each. Experiments were halted when the dissolved oxygen level in the chamber dropped below 70% of saturation. In order to approximate resting metabolic rates (i.e.,

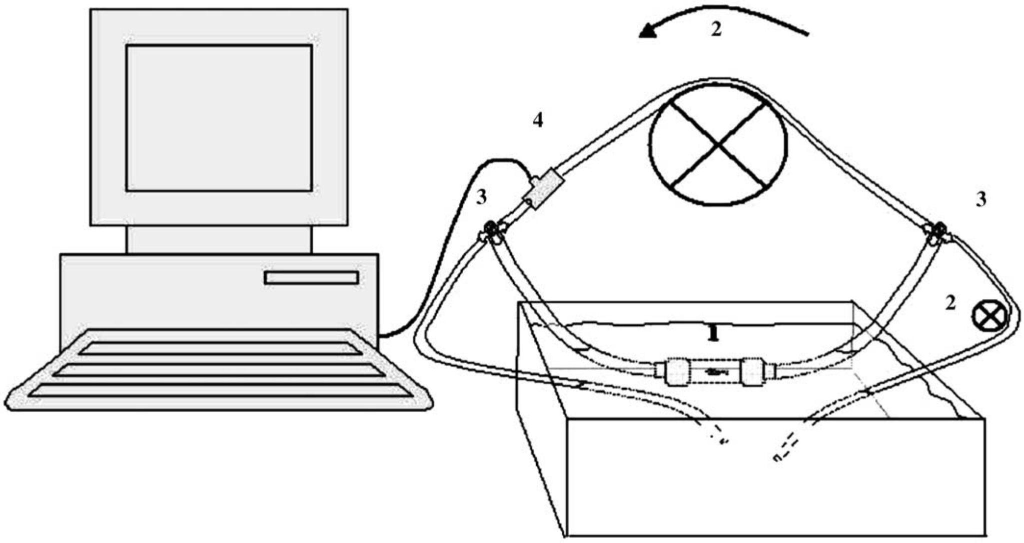

Fig. 1. Diagram of the respirometer used: (1) fish compartment; (2) peristaltic pump; (3) three-way valve; (4) flow-through fiber-optic micro oxygen sensor.

minimal activity), trials were carried out at night and in the dark. Larval behavior in the chambers was videotaped with an infrared-sensitive video camera placed underneath the chambers.

At the end of the respiration measurements, larvae were anesthetized with MS 222 and their TL (mm) measured to estimate their dry weight (DW, mg) using the equation:

$$\mathrm{DW} = 0.0005 \times \mathrm{TL}^{3.466} \quad (R^2 = 0.984)$$

This equation was derived from 90 red drum larvae of 3.3–13.5 mm TL dried at 65 °C for 24 h. Respiration rate was expressed as μg of O_2 min^{-1} mg^{-1} dry weight.

Larval activity within the chambers was quantified for a random sample of 21 fish (7 per treatment). Measurements were performed after the 2-h acclimation period. Percent of time active was measured in 30-s video segments by recording the total time spent swimming.

2.7. Statistical analyses

Statistical analyses of the data were conducted using SYSTAT software (Version 10.0). All variables were tested for normality and square root, logarithmic, or arcsine transformations were applied when necessary as described by Zar (1999). Variability between spawns was accounted for by introducing “spawn” as a blocking variable in all statistical models. Behavioral data were analyzed for each experimental day using one-way analysis of variance (ANOVA). Pairwise comparisons of treatments were performed as appropriate using the Tukey–Kramer HSD test. Predator exposure experiments generated binomial data; therefore, comparisons were conducted using contingency-table analysis and Pearson Chi-square statistics. Growth rates were compared on $\log_e$-transformed TL data using analysis of covariance (ANCOVA), with experimental day as the covariate.

3. Results

Chemical analyses revealed that 96 h after addition of atrazine to the experimental tanks, an average of 17.7% (±10.4 S.E.) of the parent compound had degraded to desethyl-atrazine (Table 1). No other degradation product was detected.

Survival of 7 mm TL red drum larvae at any of the three concentrations of atrazine (40, 80, and 500 μg l^{-1}) for 96 h was not significantly different from the control groups (averaged 77.4 ± 3.2% S.E.).

Table 1
Nominal and actual concentration in the experimental tanks 0 and 96 h after atrazine addition (±1 S.E.)

Treatment	Nominal concentration ($\mu g\,l^{-1}$)	Actual concentration 0 h ($\mu g\,l^{-1}$)	Actual concentration 96 h ($\mu g\,l^{-1}$)
Control	0	nd	nd
Low	40	37.43 ± 5.71	33.33 ± 0.8
High	80	80.51 ± 1.21	58.71 ± 10.99

nd: below the detectable level.

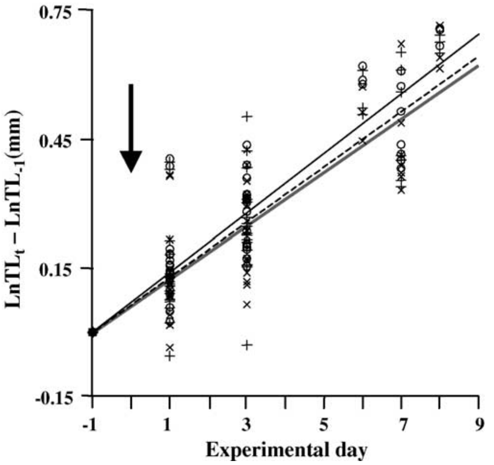

Fig. 2. Growth of red drum larvae from a common initial TL over 9 experimental days. Lines represent linear regression fit to the data grouped by treatment. Control group (◯, –); low dose group (×, - -); high dose group (+, –) (n = 59). Arrow represents atrazine addition to the experimental tanks (see text for details).

Therefore, environmentally realistic doses were within the sublethal range for red drum larvae. Exposure levels chosen for study were 40 and 80 $\mu g\,l^{-1}$.

3.1. Growth

Control larvae grew at a significantly faster rate than atrazine-exposed larvae (P = 0.01, Fig. 2). Pairwise comparisons indicated that control fish had a significantly faster growth rate from the high dose group but not from the low dose group (Table 2).

3.2. Behavioral experiments

Three spawns (replicates) were used for behavioral assays. Red drum larvae exposed to either 40 or 80 $\mu g\,l^{-1}$ of atrazine for 4 d showed significantly altered performance in all four behavioral traits analyzed compared to controls. Exposed larvae swam significantly faster, with a higher rate of travel (P = 0.001, Fig. 3a) and active swimming speed (P = 0.001, Fig. 3b) compared to control larvae. In addition, treated larvae were hyperactive (P = 0.006, Fig. 3c) and swam considerably more convoluted paths (i.e., lower NGDR, P = 0.002, Fig. 3d) after 4 days of atrazine exposure compared to unexposed larvae. For all the variables studied, significant differences were always observed between treated and control groups, but not between the low and high levels of atrazine exposure.

In contrast to routine behavior assays, no significant atrazine effect was observed for any of the visual startle response traits analyzed. Treated fish were as responsive to the visual stimulus as control fish (P = 0.26). Responsiveness averaged 55 ± 0.7% (±1 S.E.) on experimental day 1 and 59 ± 0.004% on day 3. The magnitude of the responses was also similar in all groups. Average (P = 0.89) and maximum response speeds (P = 0.68) on days 1 and 3 were 58.5 ± 4.2 and 204.4 ± 9.4 mm s^{-1}, respectively. On day 3, average response speeds increased to 78.3 ± 4.3 mm s^{-1}, while maximum response speed remained the same at 182.8 ± 12.9 mm s^{-1} on day

Table 2
Growth rates of red drum larvae over a 9 d period exposed to sublethal levels of atrazine and controls

Treatment	R^2	Growth rate, G (d^{-1})	95% confidence interval
Control (0 μg/l)	0.93	0.069	0.064–0.074
Low (40 μg/1)	0.89	0.064	0.059–0.070
High (80 μg/1)	0.0	0.063*	0.057–0.068

* Indicates significant difference (P < 0.05) relative to control (N = 59).

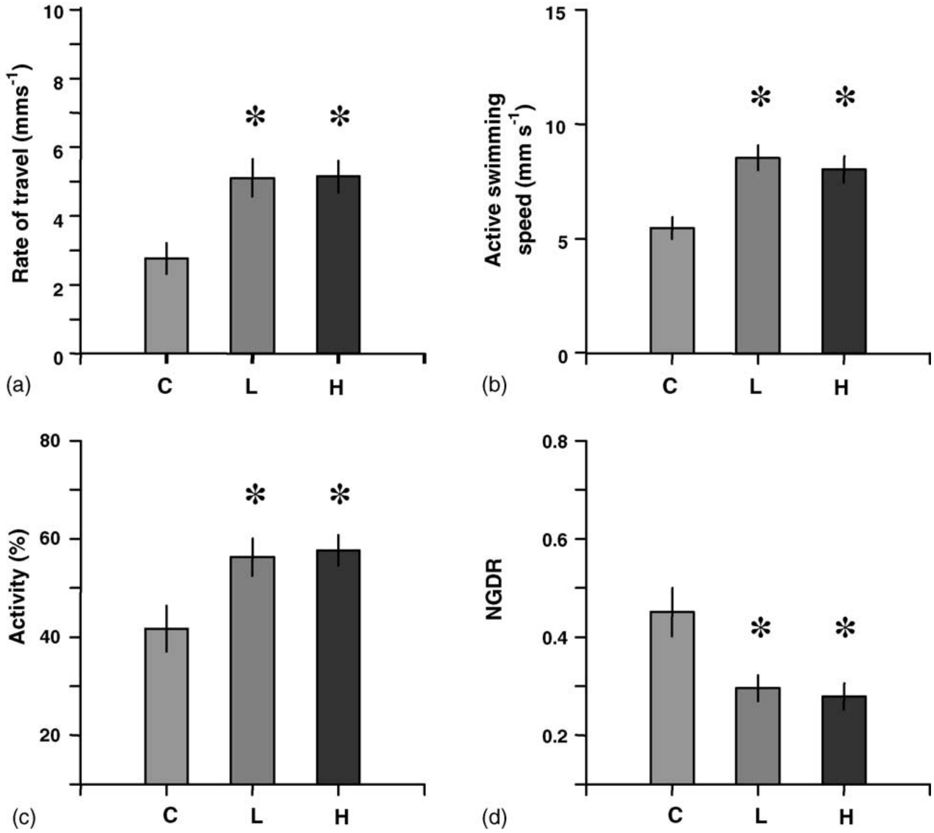

Fig. 3. Routine behaviors of red drum larvae treated with 0 (control, C), 40 (low, L), or 80 (high, H) $\mu g\,l^{-1}$ of atrazine for 4 days. (a) Rate of travel; (b) Active swimming speed; (c) activity; (d) net-to-gross displacement ratio (NGDR). Values represent means ± 1 S.E. Asterisks (*) indicate significant differences relative to controls ($P < 0.05$; $n = 36$).

3. Latencies ($P = 0.47$) and times to maximum speed ($P = 0.40$) averaged 4.4 ± 0.3 and 125.7 ± 9.0 ms for day 1, and 4.4 ± 0.2 and 115.2 ± 7.4 ms for day 3, respectively.

3.3. Predator exposure

Atrazine exposure produced no differences in antipredator performance of red drum larvae. Control and treated larvae (both exposure levels) were equally responsive to the attacking predator ($P = 0.65$). Response rates were 69.8 ± 4.3% on day 1 and 84.9 ± 1.7% on day 3 (±1 S.E.). Likewise, response effectiveness ($P = 0.31$) was high, 74.2 ± 3.6 and 82.2 ± 2.1% for days 1 and 3, respectively. These variables resulted in relatively low capture success ($P = 0.77$) for the predators, this being 44 ± 3.6% on day 1 and 29.5 ± 2.2% on day 3.

3.4. Respiration rates

Analysis of fish activity within the respirometry chambers showed that the fish were active 6.0 ± 2.4% of the time, with no significant difference among treatments ($P = 0.36$). This level of activity at night was considerably lower than the routine activity measurements made during the day (35.3–57.6%). This indicates that measurements within the respiration chamber were done on larvae that were essentially at rest. Respiration rates of atrazine-exposed fish were not statistically different from control ($P = 0.92$) and the overall average was 0.024 ± 0.001 μg of O_2 min^{-1} mg^{-1} dry weight, comparable to previously published values for red drum larvae (Torres et al., 1996).

4. Discussion

Pollutants in the environment can affect physiological processes related to growth, development, and behavior. This is especially true of compounds that disrupt endocrine function, like atrazine, since hormones and neurotransmitters are known to regulate a suite of metabolic, developmental, and behavioral pathways (Brown and Bern, 1989). Growth and behavior are crucial traits for larval survival in the natural environment. Moreover, growth is closely related to development (Fuiman et al., 1998). Atrazine, at the environmentally realistic doses used here, significantly reduced red drum larval growth rate by 7.9–9.8%, thereby, increasing the duration of the highly vulnerable larval period. Working under the assumptions that (1) atrazine effects on growth rates are permanent, or (2) atrazine exposure levels are constant during the larval period, it is possible to estimate the effects of atrazine exposure on red drum survival to the juvenile stage. Red drum larvae reach the nursery seagrass beds at about 7 mm TL and remain there past the start of the juvenile stage (Rooker and Holt, 1997; Herzka et al., 2002), which is at about 25 mm TL (based upon complete squamation; Fuiman et al., 1998). Atrazine exposure reduced growth rate by about 8.7%. Consequently, the duration of the larval period will be longer when atrazine exposure occurs. Assuming all larvae reach the nursery area at 7 mm TL, an unexposed larval cohort with a growth rate of $0.0693\,d^{-1}$ (control group) will reach the juvenile stage approximately 18 d later, whereas an atrazine-exposed cohort with an average growth rate of $0.0633\,d^{-1}$ (average of low and high dose groups growth rates) will require about 20 d to transform into juveniles. Rooker et al. (1999) estimated that instantaneous mortality rates in the seagrass beds for settled red drum larvae ranged from 0.134 to $0.139\,d^{-1}$ (average $0.1365\,d^{-1}$). This implies that a 2 d increase in larval stage duration due to atrazine exposure will increase mortality of the cohort by 24%. Such reductions in the number of juvenile fish produced in contaminated areas could potentially have a profound effect on recruitment and ultimately on the final population numbers.

Behavioral performance is critical to success in dealing with predators and prey. Although no significant effects on larval ability to evade predators were observed, environmentally realistic levels of atrazine induced changes in the routine behavior of red drum larvae. Treated larvae at both doses swam significantly faster and were hyperactive. Also treated larvae had significantly lower NGDRs than control larvae (i.e., described more convoluted paths, Fig. 4). However, since atrazine-treated larvae swam faster than control fish within the small experimental chamber, they could be expected to encounter the chamber's walls about 1.8 times more frequently than control fish. Therefore, the lower NGDRs observed for exposed fish might partially be an artifact of the confinement in the experimental chambers. Nevertheless, the other effects on routine behavior should affect a larva's likelihood of encountering predators and prey.

Routine behavior effects on rate of travel and its implications for larval survival can be estimated with the equation for encounter rates given by Gerritsen

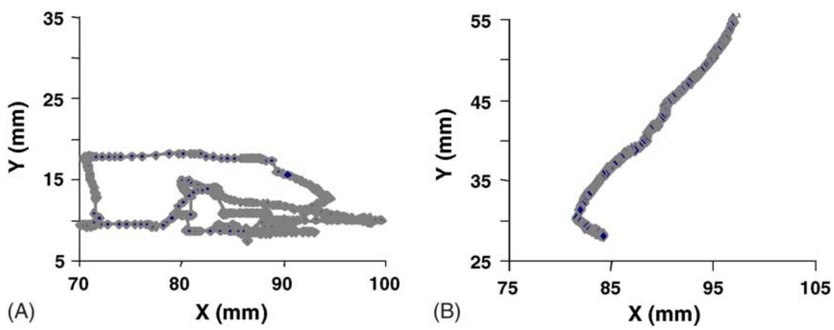

Fig. 4. Swimming paths described by a red drum larva treated with atrazine for 4 days (A) and untreated larva (B). Atrazine-exposed larvae swam a more erratic path as shown by the low NGDR (0.1) compared to control fish (0.9).

Table 3
Parameters used for calculating encounter rates of red drum larvae with prey and predators

Organism	Swimming speed ($mm\ s^{-1}$)	Average TL (mm)
Prey		
Acartia tonsa	1.42	1.0
Paracalanus parvus	1.83	0.9
Temora turbinata	1.81	1.2
Predators		
Small ctenophore	3.75	15
Small medusa	6.25	25
Large ctenophore	11.25	45
Large medusa	18.75	75
Juvenile red drum	80.9	27
Planktivorous fish	105	35
Red drum larvae		
Control	2.76	8
Atrazine-treated	5.13	8

Data for prey obtained from Waggett (2005) and for predators from Cowan et al. (1996). Encounter rates were estimated for a 24-h period in a volume of 200 m^3.

and Strickler (1977) and modified by Bailey and Batty (1983), as done by Cowan et al. (1996). Calculations were made for three species of calanoid copepods that inhabit settlement grounds: *Acartia tonsa*, *Paracalanus parvus*, and *Temora turbinata* (newly settled red drum larvae feed mainly on calanoids [Soto et al., 1999]). Encounter rates were also calculated for five predator species as described by Cowan et al. (1996), including juvenile red drum (cannibalism in red drum has been observed under laboratory conditions [Fuiman, 1994]). Parameters used in the calculations are provided in Table 3.

Although encounter rates calculated here are derived from mathematical models, and therefore, may not be entirely realistic, they can be used in a comparative sense to interpret potential effects of atrazine exposure on outcomes of predator–prey interactions. These calculations show that the apparently contradictory effects of atrazine on rate of travel are predicted to produce substantial increases in encounter rates with prey. Calculations using daily foraging distance (rate of travel 24 h) showed that exposed larvae have a greater probability of encountering prey (average increase = 71%, Fig. 5). In the same way, atrazine-exposed larvae are also more likely to encounter predators (Fig. 6), where the outcome is often fatal. Changes in rate of travel have a larger predicted influence on encounter rates with slower swimming predators, such as ctenophores and medusae. The ecological importance of encounters with gelatinous predators in the nursery areas is probably low since red drum larvae will seek cover in the seagrass beds where

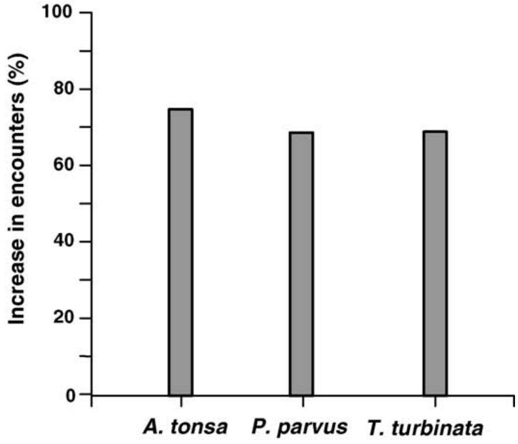

Fig. 5. Increase in predicted encounter rates (relative to controls) between red drum larvae and prey as a result of atrazine exposure for 4 days. Prey items are three types of calanoid copepods: *Acartia tonsa*, *Paracalanus parvus*, and *Temora turbinata*. Calanoid copepod speeds were obtained from Waggett (2005).

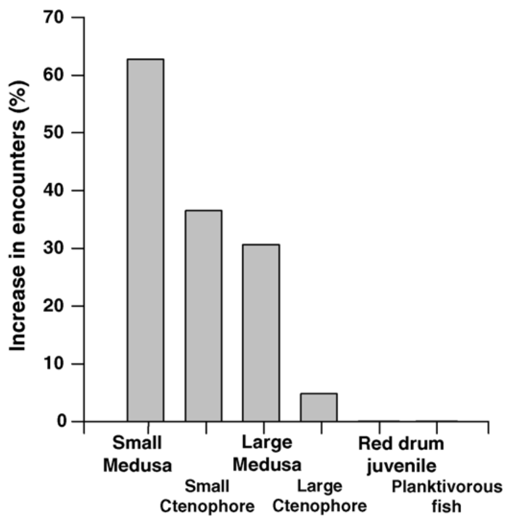

Fig. 6. Increase in encounter rates (relative to controls) between red drum larvae and their predators as the result of atrazine exposure for 4 days. Predators are: two sizes of ctenophores (small and large), two sizes of medusa (small and large), 25 mm red drum juvenile, and 35 mm planktivorous fish.

Table 4
Estimated effects of atrazine exposure on total metabolic rate of red drum larvae ($R_t = P \cdot R_a + (1 - P) \cdot R_s$)

	u	R_a	R_s	P	$P \cdot R_a$	$(1-P) \cdot R_s$	R_t	Q_{10} corrected R_t
Control	5.48	50.09	0.19	0.42	20.89	0.11	21.00	77.84
Treatment	8.28	75.19	0.19	0.57	42.82	0.08	42.90	158.85

Active respiration rate was calculated as a linear function of swimming speed (u) ($R_a = 1.13 + 8.94 \cdot u$; Hunt von Herbing et al., 2001). R_s: resting metabolic rate (mg O_2 g^{-1} h^{-1}); P: proportion of time active; R_a: active metabolic rate: and R_t: total metabolic rate. R_t was adjusted for differences in temperature using Q_{10} values of 1.9 for R_a and 2.5 for R_s (Kaufmann and Wieser, 1992).

this type of predator is uncommon and ineffective. However, during the time the larvae are entering the estuary and still in a pelagic stage, an increase in encounters with this kind of predator can have a profound effect on the total number of larvae colonizing the seagrasses. Predatory fish (planktivorous and red drum juveniles) used for these calculations swim much faster than larvae, thus contaminant effects on red drum larvae will not result on detectable changes in encounter rates.

However, another consideration that is not included in the encounter-rate calculations is that red drum larvae exposed to atrazine for 4 d were 20% more active than unexposed larvae. Seagrass nurseries offer shelter from predators to the young larvae. However, it is expected that hyperactivity in treated larvae will make them more conspicuous to visual predators than control and hence increases the probability of being captured.

Respiration rates are good estimates of total metabolic rates since fish larvae rely mainly on aerobic metabolism (Finn et al., 1995; Wieser, 1995; Brightman et al., 1997). Measurements of respiration rates were intended to determine whether larvae exposed to atrazine incurred a direct metabolic or energetic cost. Thus, our respirometry trials were designed to minimize larval activity (measurements were made in the dark at night on postabsorptive larvae) so that measurements approximated a resting or standard metabolic rate.

For unfed animals, total metabolic rate (R_t) is the weighted sum of active (R_a) and standard (R_s) (or resting) metabolic rates:

$$R_t = P \cdot R_a + (1 - P) \cdot R_s$$

where P is the proportion of time active. Active respiration rate (R_a) is primarily a function of swimming speed (u). This relationship is an exponential function when larvae are in an inertial hydrodynamic regime, moving at relatively high Reynolds numbers (Re) where drag is proportional to the square of u [e.g., cyprinid larvae (Kaufmann, 1990)]. In the viscous hydrodynamic regime (low Re), the relationship is linear since drag is directly related to u. Larvae 4 days in the experiment exhibited an average Re of 58. Since viscous effects extend to Re numbers of 300–450 (Fuiman and Batty, 1997) a linear relationship is appropriate. Hunt von Herbing et al. (2001) described this equation for Atlantic cod (*Gadus morhua*) larvae:

$$R_a = 1.13 + 8.94 \cdot u$$

where R_a is in mg O_2 $g^{-1}\,h^{-1}$ and u is in mm s^{-1}. In the current study of red drum larvae, R_s averaged 0.19 mg O_2 $g^{-1}\,h^{-1}$. Calculated R_t of treated fish was double that of control fish (Table 4). However, since cod larvae exist at substantially lower water temperatures (about 20 °C lower than red drum in our experiment) and metabolic rates are dependent upon temperature, metabolic rates were adjusted using Q_{10} values of 1.9 for R_a and 2.5 for R_s (Kaufmann and Wieser, 1992). These adjustments did not affect the results; R_t for atrazine-treated larvae was double that of control R_t (Table 4).

No significant differences were observed between the two levels of atrazine exposure (40 and 80 μg l^{-1}) in any of the variables tested here. Low and high dose groups were equally disruptive to red drum larvae compared to control in all but one of the variables investigated (i.e., growth).

5. Conclusions

Endpoints for assessing sublethal effects of pollutants can be obtained at almost any level of biological organization: molecular, cellular, tissue, organ. The higher the level affected by a pollutant the more generalized the response and the greater the implications for the general health of the organism, population, and ecosystem. Many studies in aquatic toxicology focus on single endpoints at one level (Weis and Weis, 1989; Saglio and Trijasse, 1998; Kazeto et al., 2004). However, few studies analyze effects at different endpoints (Beauvais et al., 2000; Brewer et al., 2001; Zhou et al., 2001). Moreover, studies often use contaminant levels and/or modes of exposure that do not reflect environmentally realistic conditions for the organism under study. Although such studies are important for characterizing the toxicity of a compound, it is difficult to use them to understand the ecological impacts of pollution. Scott and Sloman (2004) recognized the lack of studies evaluating the implications of interrelated behavioral and physiological effects caused by aquatic pollutants for fish populations. In the present study, we used a comprehensive approach that allows us to make detailed predictions for the outcome of ecologically relevant and sublethal exposures to atrazine for red drum larvae.

Environmentally realistic levels of atrazine are not directly lethal for red drum larvae although they could pose a threat to survival through effects on growth and behavior. Reduced growth will increase mortality by prolonging the highly vulnerable larval period. Alterations of routine behavior caused by atrazine exposure may produce a considerable foraging advantage for treated larvae by increasing encounter rates with prey, but the same effects on routine behavior produce a very high energetic burden (a doubling of R_t). Exposed larvae will need to meet this elevated energetic requirement or suffer reduced growth and possible starvation. Predicted increases in encounter rates with prey averaging 71% might not be enough to cope with this energetic burden in the patchy and highly variable natural environment. Moreover, encountering prey does not guarantee ingesting and absorbing the required nutrients. In these experiments, fish were fed high rations yet an effect on growth was observed. This suggests that one or more processes between encountering prey and growth, such as capture, ingestion, digestion, or protein metabolism, was impaired.

Finally, behavioral effects of atrazine exposure also increase the probability of encountering slow moving predators (ctenophores and medusa), which can result in an increased mortality during the period larvae are entering estuarine nursery areas.

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